Transcranial Doppler Ultrasound in Pediatric Neurocritical Care: Clinical and Research Applications

Genetic Markers Associated with Cerebral Vasospasm in Pediatric TBI

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Disclosures

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- The content of this presentation does not contain reference to, nor advocates use of, unlicensed medicines or devices.

Objectives

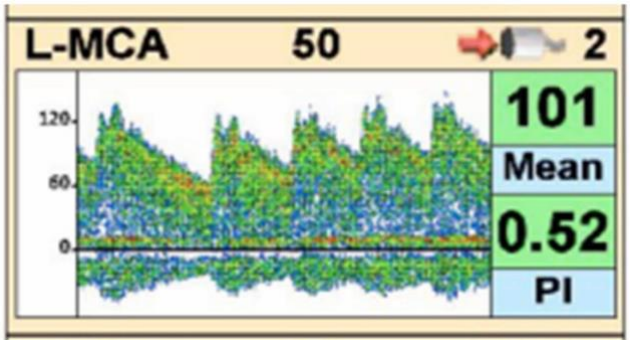
- The use of TCD in pediatric TBI research.
- Study of genetic markers and their relationship to cerebral vasospasm in children with TBI.

Traumatic Brain Injury (TBI)

- 1/3 of all injury-related deaths in the U.S.
- *Leading cause* of morbidity & mortality in children & youth
- Mortality highest among the young, boys, & minorities
- Personal, financial, & societal costs of TBI are immense
- Limited therapeutic interventions currently available make this an area of compelling clinical need
- Diagnostic genetic markers may clinically identify those at risk for poor outcomes

TCD Use in Pediatric TBI Research

- Cerebral Vasospasm (CV) is a 2ndary injury phenomenon
  - Leads to cerebral hypoperfusion, ischemia, vascular insults
- Children with mod-severe TBI found to experience CV O'Brien, Reuter-Rice et al. 2010; O'Brien et al., 2015
- The prevalence of CV in peds TBI is 21% (anterior circulation) and 12% (posterior circulation) O'Brien et al., 2015
- CV following TBI:
  - Time of onset & duration
  - Poor neuro-functional outcomes



Study Participant Criteria

INCLUSION:

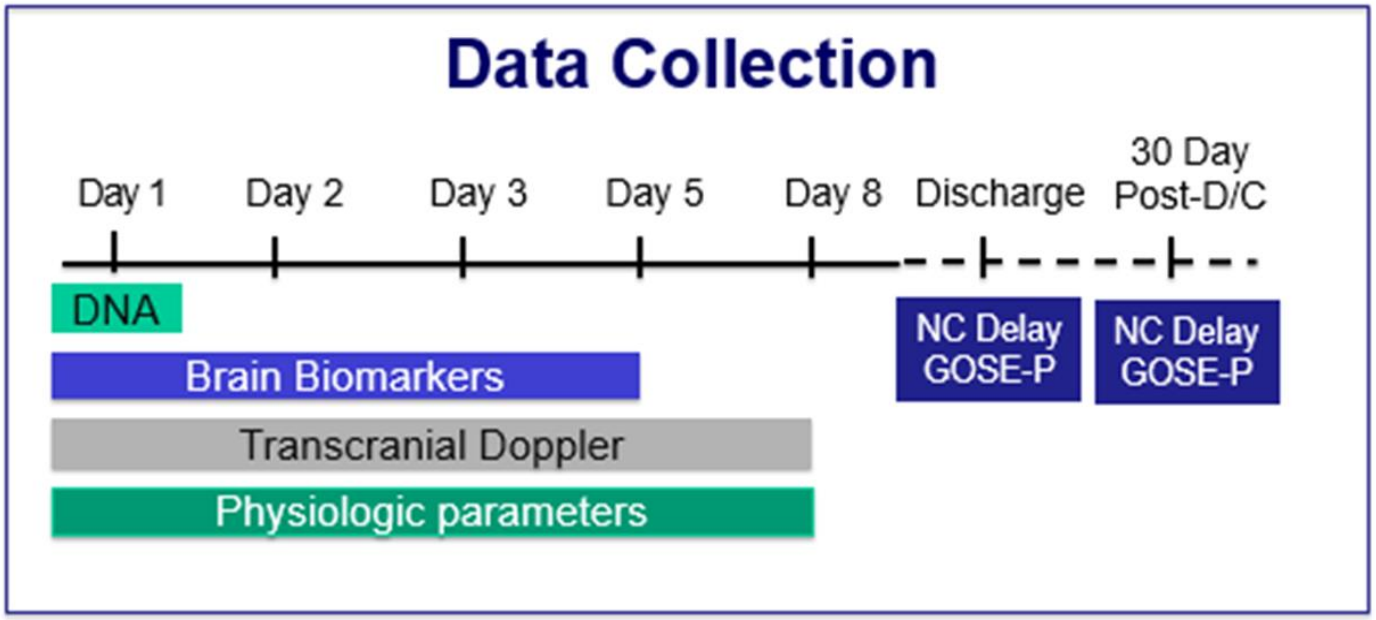
- Previously healthy
- 5 days to 15 years
- Admitted to DUHS with a TBI
- Under standard of care treatment
- GCS 3 to 15
- Capable of adequate TCD ultrasound

EXCLUSION:

- Presence of previous neurodevelopmental delay
- Diagnosis of non-traumatic intracranial hemorrhage

Study Design

- 3 year prospective exploratory study (2013-2015)
- *N* = 60 children admitted for TBI to DUHS
  - Presence of CV was determined by using Transcranial Doppler ultrasound (Sonara Digital TCD, Natus)



Biologic Analyses

- Genotyping by TaqMan® allele discrimination assays for the presence of genetic markers
  - Genetic markers (Apolipoprotein E [*APOE*], Endothelin 1 [*EDN1*])

- Additional protein analyses performed by Astute Medical Inc, San Diego CA
  - Neuroinflammatory associated biomarkers:

    GFAP, CKBB, NSE, S100B, CRP, IL6, ET-1

TCD Timing and Measures

- TCD within 24hrs of admission for CBFV & LR Aaslid et al. 1982; Bode & Wais, 1988; O'Brien, 2015

High MCA flow velocity	
V_{mca}	$\geq$ 2 SD above normal age value
CV (V_{mca} + LR)	
V_{mca}	$\geq$ 2 SD above normal age value
LR	$V_{mca} : V_{EC\text{-}ICA} \geq 3$
BA vasospasm	
Vba	$\geq$ 2 SD above normal age value

Reuter-Rice, *J Radiol Nurs*. 2017 Mar; 36(1): 3–9.

- Glasgow Outcome Scale-Extended Pediatrics Beers et al. 2012
  - *at discharge (T1) and 4-6 weeks post discharge (T2)*

Parent Study Sample Characteristics

Characteristics		Results (N=60)
Admission location, (n) %	Pediatric ICU	(40) 67
	Non-ICU	(20) 33
Gender, (n) %	Male	(34) 57
	Female	(26) 43
Mean Age, years		5.5
Race, (n) %	African American/ Black	(20) 33
	Caucasian/White	(37) 62
	Other	(3) 5
Ethnicity, (n) %	Non-Hispanic or Latino	(53) 88
	Hispanic or Latino	(7) 12
Glasgow Coma Scale (GCS), (n) %	Mild (GCS 13-15)	(44) 73
	Moderate (GCS 9-12)	(2) 3
	Severe (GCS 3-8)	(14) 24
Mechanism of Injury, (n) %	Fall	(23) 39
	Abusive Head Trauma	(18) 30
	Motor vehicle related	(7) 11
	Other/recreational	(12) 20
Diagnosed Injury, (n) %	Subdural	(27) 45
	Epidural	(13) 22
	Subarachnoid	(13) 22
	Other	(7) 11
Mean Length of Stay, days		9.5

Represents expected population:

- Boys > girls
- 38% non-white
- Strong representation of mild & severe
- Majority falls
- High # subdural injury
- Mean LOS ~10 d

Study #1:

The Effect of the Relationship of APOE Polymorphisms and Cerebral Vasospasm on Functional Outcomes in Children with Traumatic Brain Injury

Biol Res Nurs. 2018 Oct;20(5):566-576

Karin Reuter-Rice, PhD, NP, Michael Regier, PhD, Ellen Bennett, PhD, & Daniel Laskowitz, MD, MS

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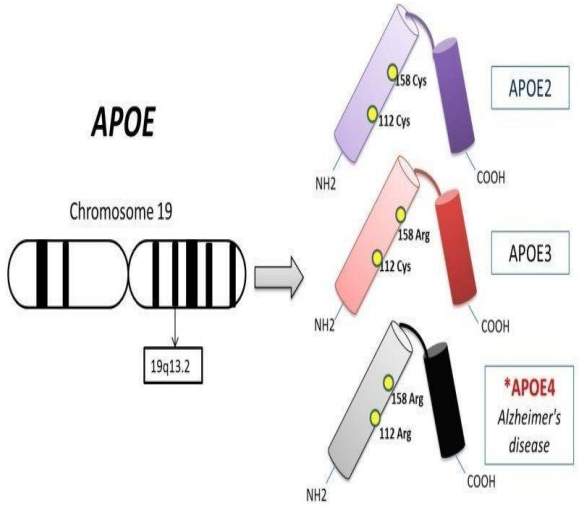
Apolipoprotein E (*APOE*)

- *APOE* is a protein coding gene
- 3 common protein isoforms

  APOE2, APOE3, APOE4



- SNP rs405509, results in a G/T nucleotide change at position -219 and modifies APOE gene expression Bennett, Reuter-Rice, Laskowitz, 2016
- A study of 185 adult with SAH found the presence of the APOE4 allele was a risk factor for CV Wu et al. 2010

APOE & CV

- Apolipoprotein E (*APOE*) has been link to CV and poor outcomes in adults with TBI Lee et al. 1997; Oertel et al. 2005
- APOE4 allele is associated with poorer outcomes in children with TBI Teasdale et al. 2005; Brichtová et al. 2008; Kassam et al. 2016

No pediatric TBI studies examining *APOE4* SNPs and CV

AIM: Examine the relationship between the *APOE*, *specifically APOE4* SNPs (rs405509, rs429358, rs7412) and CV to neuro-functional outcomes in children with TBI.

CV Incidence & Genotypes

- CV incidence 43.3% (n=26)
  - *Anterior circulation: n=5 vs. Posterior circulation: n=25*
  - *Males 54% vs. Females 46%*
  - *Most prevalent with hx of falls (42%)*
  - *Most prevalent in young <6yo (65%)*

APOE genotype	No CV	Yes CV	p=0.649
E2E3	3 (8.8)	5 (19.2)	
E3E3	23 (67.7)	16 (61.5)	
E3E4	7 (20.6)	5 (19.2)	
E4E4	1 (2.9)	0 (0)	

APOE Study Findings

- There were significant differences in injury mechanism (unadjusted $p = 0.048$) and age (unadjusted $p = 0.02$) between those with and without CV
- The noncoding promoter SNP rs405509 T/T, when considered with injury severity, appeared to modify the relationship of *APOE* genotype to CV
- The relationship between APOE and CV had no significant effect on GOS-E Peds scores
- Higher incidence of CV in posterior circulation

Study #2:

Endothelin 1 Gene Variant May Play a Role in Cerebral Vasospasm in Children with Traumatic Brain Injury

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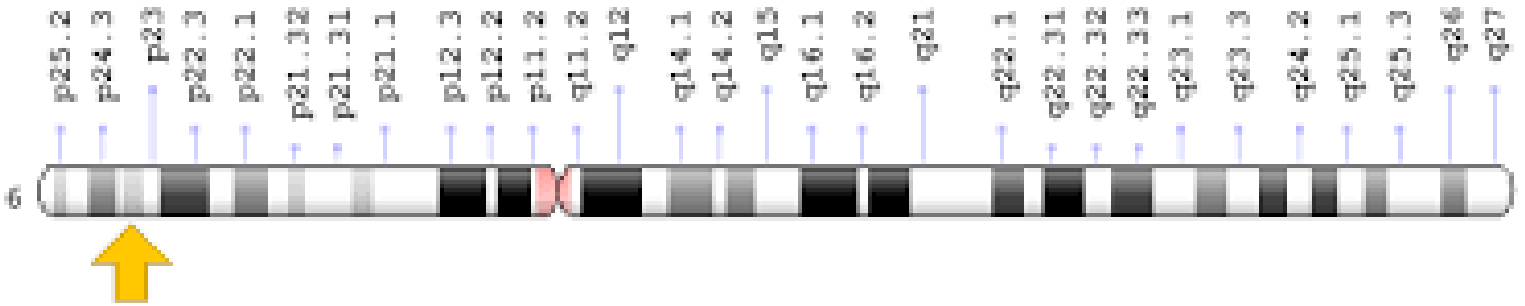
Endothelin 1 (*EDN1*)



- Chromosome position: 6p24.1
- ***EDN1*** gene encodes **ET-1 protein**, a potent vasoconstrictor
- rs2070699 (G>T or C) single nucleotide polymorphism (SNP)
- rs5370 (G>T) confers functional change in EDN1 = Lys198Asn
- Associated with CV in SAH Gallek, 2008

EDN1 & CV

- Animal model TBI (fluid percussion injury): changes in cerebral hemodynamics associated with neuronal damage

- Model implicated *EDN1* as a *possible role for CV:*

ET-1 → ↑ Intracellular Ca^{++} → calpain → CV

Armstead & Raghupathi, 2011

- Genetic variation in *EDN1* may account for the variance observed in functional outcomes in adult SAH Arutiunov et al. 1975; McGirt et al. 2002 Siman et al. 2011
- Trend found between *EDN1* SNP rs6912834 & angiographic vasospasm

EDN1 & CV

- Children with severe TBI showed ↑ levels of ET-1 over time
  - *Associated with poor GOS & functional outcomes*
  - Patients had sustained vasoconstriction & ↓ cerebral blood flow ***suggestive of vasospasm*** (TCD not measured) Salonia et al. 2010

No pediatric TBI studies examining *EDN1* SNPs and CV

AIM: *EDN1* rs5370, rs2070699 association with CV in pediatric TBI

CV Incidence by Circulation, Injury Severity, & Lesion

- Overall CV 43% incidence (n=26)
  - Anterior circulation (n=5)
  - Posterior circulation: (n=25)
    - Children with *mild* TBI (all ages) had highest incidence
  - Subdural hemorrhage associated with the highest incidence (52%)

EDN1 SNP Study Findings

- Of the 60 children, 85% (*n*=51) had genotype data (*n*=9 missing data)
- Children with any copy of the **rs2070699 (T)** risk allele (60%) showed trend towards greater incidence *of CV* compared to G/G homozygous carriers (*p*=0.07)
- No significant difference in **rs5370 (T)** risk allele carrier status (40%) between CV positive and CV negative children (*p*=1.0)

Genetic Implications in Pediatric TBI Research

- **APOE4 SNP rs405509 *may modify*** the relationship between APOE and CV in children with TBI
- *More studies are needed; examine the age effect of APOE*
- First to report evidence of *gene association trend* with presence of ***EDN1* SNP rs2070699 risk allele (T)** and ***incidence of CV*** among children with TBI
- *More studies to compare these findings in adults with TBI*
- Candidate *EDN1* and *APOE4* demonstrate limited evidence Reuter-Rice et al. 2018
- *Novel marker discovery needed for future powered research*

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